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phenotypes may nonrandomly assort in space or time, such that pairs primarily encounter and mate with their own type. Dingemanse *et al.* consider only mechanism (i) to be relevant to mate choice by BT. We take a broader view. While classic mate choice is often thought of as active selection ('I want that one'), because we perhaps never know what an organism is thinking, mate choice can be more objectively defined as a behavior of the chooser that enhances the probability of mating with some phenotypes over others.

Consider a two-phase process: situation choice, followed by classic mate choice. Situation choice can involve choice of habitat, social group, or active times during the mating season. When any one of these depends on BT [5], the result can be a tendency for individuals to encounter more individuals of their own BT. For instance, if bold versus shy individuals tend to choose different habitats, each will likely end up near others of similar BT. Even with random mating within habitats, pooling across multiple habitats, the outcome can be BT-dependent assortative mating. While this mechanism generates assortative mating with no classic mate choice, the situation choice per se fits the objective definition of mate choice. It is behavior that results in nonrandom mating. In particular, if during the mating season, animals prefer a particular situation in part because of the BT of potential mates they expect to find there, then situation choice clearly, in part, reflects mate choice.

Individuals may also adjust their BT to fit the situation, such that even with no BT-dependent situation choice, individuals in a shared environment express similar BTs by the time they mate. If mating with a similar partner that is well adapted to the current environment improves reproductive success [6], then the tendency for one or both sexes to adjust their BT to their shared environment represents adaptive plasticity that enhances the likelihood and success of mating with partners with a similar BT. This mechanism is more likely to be important if males and females live together for long enough for adjustment of BTs. In contrast, if males and females live in other habitats prior to mate choice, or if their BT is shaped by factors other than current environment, then the shared experience effect is likely less important.

Following situation choice, individuals choose a mate among those they encounter (which again may be BT dependent [7]). If individuals choose a mate that is phenotypically similar to themselves, this is assortative mating by classic mate choice. Following mating, individuals may adjust their BT to become more similar [8]. Again, if behavioral synchronization is beneficial, selection favoring pairs that have ultimately end up assorted by BT could explain the mate-dependent BT plasticity.

Dingemanse *et al.*'s call for more rigorous testing of assortative mating is well taken. Understanding how much the patterns we see in mate choice are due strictly to classic choice for phenotypically (dis)similar mates and how much is due to: (i) situation choice and adjustment; and (ii) perhaps most interestingly, how much situation choice and adjustment are shaped by the benefits of assortative mating, will only improve our understanding of assortative mating as a whole. However, this requires careful experimental design and rigorous analysis. To emphasize though, we suggest that to understand patterns of mate choice and BT, all of the above processes can be important.

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Forum

Unraveling the Zoonotic Origin and Transmission of SARS-CoV-2

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The origin and zoonotic transmission route of SARS-CoV-2 remain speculative. We discuss scenarios for the zoonotic emergence of SARS-CoV-2, and also explore the missing evidence and ecological considerations that are necessary

to confidently identify the origin and transmission route of SARS-CoV-2 and to prevent future pandemics of zoonotic viruses.

Speculations about the Origin of SARS-CoV-2

In December 2019 a human outbreak of pneumonia, later named coronavirus disease (COVID-19), began spreading across the planet, infecting millions of people. The causative agent of COVID-19 was quickly identified as a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Some of the earliest cases of COVID-19 in Wuhan clustered around a local seafood market, giving rise to speculations about an animal origin for the virus, with the possibility that it may have crossed the species barrier to infect humans (**zoonotic event,** see Glossary) in the market [1].

Earlier this year, researchers in China isolated and sequenced the genome of SARS-CoV-2 [1,2]. On comparison with other CoV sequences, two bat SARS-related coronaviruses (SARSr-CoV), RaTG13 and RmYN02, were identified as the closest known ancestors of SARS-CoV-2. RaTG13, identified in intermediate horseshoe bats (*Rhinolophus affinis*) in Yunnan, China exhibits 96.2% sequence identity to SARS-CoV-2 at the whole-genome level and clusters with SARS-CoV-2 in **phylogenetic analysis** [1]. RmYN02, identified in Malayan horseshoe bats (*Rhinolophus malayanus*) from Yunnan is 93.3% identical to SARS-CoV-2 at the whole-genome level [3]. These SARSr-CoVs in bats could represent the evolutionary source of SARS-CoV-2. Alternatively, SARSr-CoVs detected in bats could represent a clade that evolved independently from a common ancestor of SARS-CoV-2. We discuss recent data that shed light on possible zoonotic origins of SARS-CoV-2, and speculate on evolutionary and spillover scenarios that may have played a role in its emergence.

Evidence Hinting at a Bat Origin

Although close evolutionary relationships to bat CoVs suggest a bat origin for SARS-CoV-2, our understanding is notably limited by the scarcity of available sequenced CoV genomes. These genomes represent a mere fraction of the natural CoV diversity. One may conclude that closer relatives to SARS-CoV-2 are likely to exist, but have not yet been sequenced, which raises the question – is 96.2% genomic identity between strains sufficient to confidently identify a reservoir host? For example, palm civets (*Paguma larvata*), the likely animal source of the SARS outbreak in 2002–2003, carried a SARS-CoV-related virus that was 99.8% identical to SARS-CoV [4]. CoVs highly homologous to SARS-CoV-2 have not been identified in any animal host, but detection of SARS-CoV-2 in an animal species in the future could be confounded by the possibility of **zooanthroponosis**. Recent phylogenetic analyses indicate that SARSr-CoVs likely diverged from an ancestral bat-derived CoV between 1948 and 1982 [5], suggesting that SARSr-CoVs have been circulating in selected bat species for some time. The order *Chiroptera* represents >1400 species of bats, and emerging theoretical and experimental data suggest that not all bat species may support SARS-CoV-2 replication [6]. It is also possible that a SARSr-CoV evolved into SARS-CoV-2 in humans after spilling over from an animal source, followed by rapid transmission of this human-adapted strain [7]. Theories on laboratory escape of existing SARSr-CoVs have no valid supportive evidence. Despite these speculations, the transmission route of SARS-CoV-2 or SARSr-CoV from bats to humans, either directly or through an intermediate animal species, remains elusive (Figure 1).

Are Pangolins Intermediate Hosts?

Two independent studies identified SARSr-CoVs in confiscated Malayan pangolins (*Manis javanica*), whereas confiscated Chinese pangolins (*Manis pentadactyla*) tested negative [8,9] (Table 1). Both Lam and Xiao *et al.* isolated SARSr-CoVs from pangolins that were confiscated during illegal wildlife trade. Importantly, CoVs isolated from these pangolins were only 85.5–92.4% similar to SARS-CoV-2 at the whole-genome level [8], but possess intriguing similarities to SARS-CoV-2 in regions that are crucial for interaction with the human cellular receptor, angiotensin-converting enzyme 2 (ACE2).

The receptor-binding domain (RBD) within the CoV spike protein makes key contacts with ACE2 to facilitate viral entry. One pangolin SARSr-CoV RBD is 97.4% identical to the SARS-CoV-2 RBD, suggesting that SARS-CoV-2 may have either acquired the RBD from pangolin CoVs via recombination or developed this similarity through **convergent evolution** [8,9] (Figure 1). CoVs are prone to recombination, but a recent study reported an absence of any evidence for recombination in the spike proteins of CoVs in the lineage leading to SARS-CoV-2 and other related *Sarbecoviruses* [5]. Thus, the origin of a pangolin SARSr-CoV-like RBD in SARS-CoV-2 remains a mystery (Figure 1).

Intermediate hosts play an important role in the amplification and adaptation of zoonotic viruses. An ideal intermediate

Glossary

Adaptive evolution: the accumulation of advantageous mutations while propagating in a host.

Convergent evolution: the independent evolution of similar mutations or traits in different species.

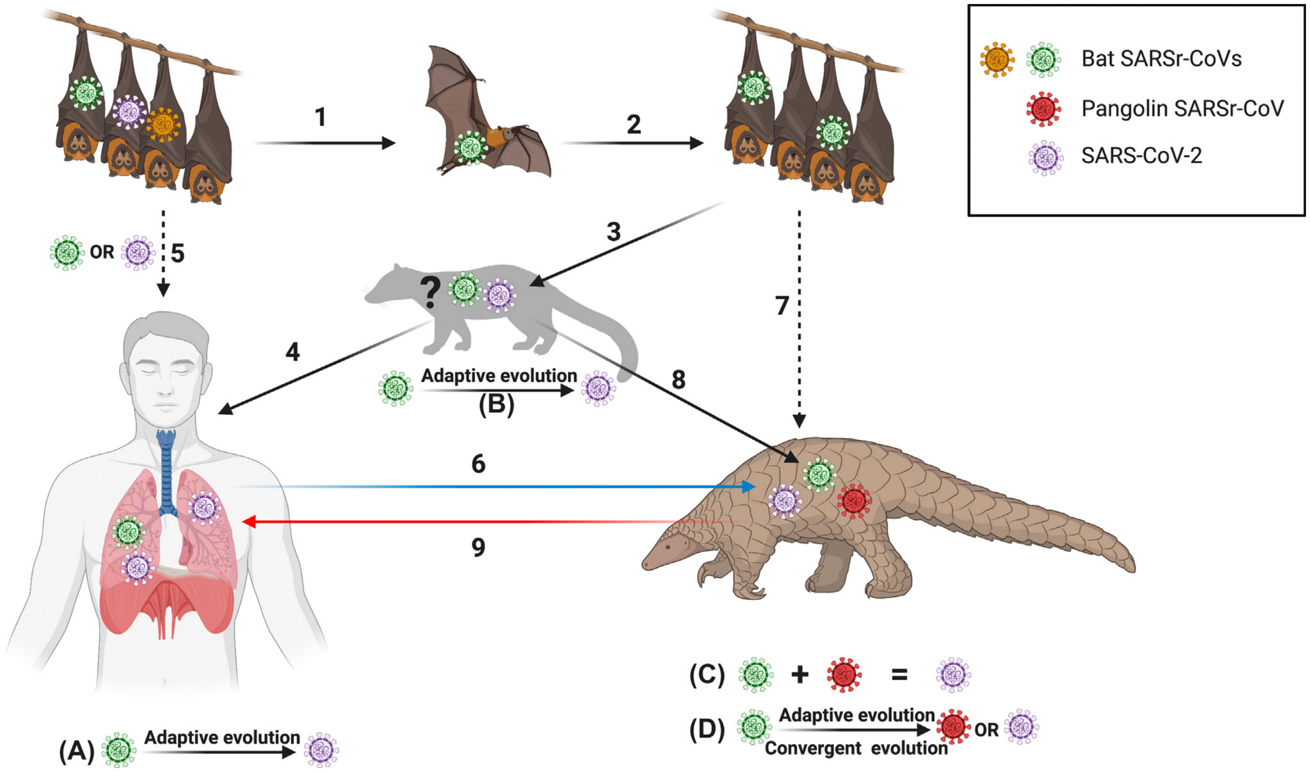
Enzootic: the presence of disease within animal populations in a region.

Koch's postulates: four criteria for establishing a causal relationship between a pathogen and a disease.

Phylogenetic analysis: the study of evolutionary relationships.

Zooanthroponosis: human-to-animal transmission of a disease or pathogen.

Zoonotic event: transmission of a pathogen from animal to humans.



Trends in Ecology & Evolution

Figure 1. Possible Scenarios for the Origin of SARS-CoV-2. SARS-related coronaviruses (SARSr-CoVs) have been circulating in selected species of bats. It is possible that SARS-CoV-2 remains undiscovered in bats (1). Bats may spread CoVs within bat populations without causing clinical signs of disease (2). Owing to unknown factors, bats may occasionally shed CoVs. Bat SARSr-CoVs may infect humans directly (5) or via an intermediate host (3 and 4), and select for human-adapted strains such as SARS-CoV-2 through **adaptive evolution** (5; scenario A). A bat SARSr-CoV could have evolved into SARS-CoV-2 in bats before spilling over into humans (5). Alternatively, bat SARSr-CoVs may infect other mammalian intermediate species that remain to be discovered (3) (? indicates an undiscovered intermediate host), and the virus may undergo adaptive evolution in these animals (scenario B). Virus species with enhanced fitness, such as SARS-CoV-2, could then infect humans that are in close contact with the intermediate animal host (4). Pangolins could have been infected with a bat SARSr-CoV, either directly (7) or via an undiscovered intermediate host (8), leading to recombination events between existing pangolin SARSr-CoVs and bat SARSr-CoVs to generate SARS-CoV-2 (scenario C). The recombined virus could have then spilled over into humans (9). Alternatively, pangolins could have been infected with SARSr-CoVs from bats (6 or 7), followed by adaptive or convergent evolution (scenario D) to generate pangolin SARSr-CoVs and/or SARS-CoV-2. Figure created with BioRender.com.

reservoir host does not develop severe disease on infection with a virus, a key feature that allows a virus to multiply and seek alternative hosts without killing its evolutionary host. However, SARSr-CoV-infected pangolins in two studies demonstrated clinical signs of disease, including histological changes and severe respiratory disease (Table 1) [9,10], observations that are inconsistent with pangolins being a successful intermediate reservoir. Because the sampled pangolins were either dead or extremely sick when specimens were collected (Table 1), and **Koch's postulates** have not been established for SARSr-CoVs in pangolins, it is possible that other underlying factors produced disease symptoms in these animals. The presence of disease symptoms in infected pangolins is further complicated by the likely presence of other viruses such as Sendai virus [11]. The sampled pangolins could have also been exposed to CoVs by other animal species or humans along the wildlife trade route [12] (Figure 1). Recent data from surveillance of 334 Malayan/Sunda pangolins (*Manis javanica*) failed to detect CoV genetic material, raising further doubts about the role of pangolins as natural intermediate hosts of SARS-CoV-2 [13].

There is evidence suggesting that particular species of pangolins (*Smutsia gigantea* and *Phataginus tricuspis*) and bats (*Hipposideridae* spp., *Emballonuridae* spp. and *Miniopterus* spp.) cohabitat in a natural setting, such as in underground caves, which may facilitate the exchange of CoVs, although there was no evidence of CoV infection in this study [14] (Figure 1). Rhinolophid bats and pangolins also share some dietary overlap (e.g., termites), which may facilitate exchange of viruses; however, direct transmission via insects is unlikely. Indeed, full examination of bat and pangolin CoV susceptibility, species

Table 1. Detection of SARSr-CoVs in Pangolins

Common name	Species	Number positive for CoV	Tissue or sample tested	Source	Percent sequence identity to SARS-CoV-2 (whole genome)	Health status	Refs
Chinese pangolins	*Manis pentadactyla*	0/4	Lung	Wildlife rescue center, Guangdong	No CoV detected	Not examined	[9]
Malayan pangolins	*Manis javanica*	17/25[a]	Lung	Wildlife rescue center, Guangdong	81.60%	Respiratory disease with alveolar damage, emaciation, lack of appetite, inactivity	[9]
Malayan pangolins	*Manis javanica*	5/18	Lung, intestine, blood	Guangxi customs office	85.5–92.4%	Dead	[8]
Malayan pangolins	*Manis javanica*	2/11[a]	Lung, lymph, spleen	Guangdong wildlife rescue center	Not compared at the time	Dead	[11]
Malayan pangolins	*Manis javanica*	3/27[a]	Lung, lymph, spleen	Guangdong wildlife rescue center	90.32%	Severe respiratory disease	[10]
Malayan pangolins	*Manis javanica*	0/334	Throat and rectum swabs	Peninsular Malaysia; Sabah, Malaysia	No CoV detected	Weak only	[13]

[a] Overlap between samples.

dependencies, and their ecological overlap will be needed before drawing conclusions about **enzootic** transmission cycles.

Interconnectedness of Ecosystem Health and Virus Spillover

There are currently only speculations about the origins of SARS-CoV-2, and direct evidence is lacking. Genetic evidence suggests that birds are the ancestral source of *Delta-* and *Gammacoronaviruses*, whereas bats are the original source for all *Alpha-* and *Betacoronaviruses*. However, it remains uncertain whether a bat species was only involved in the evolution of SARS-CoV-2 or also mediated direct bat-to-human transmission. Identifying the wildlife source of SARS-CoV-2 will help to prevent future and/or ongoing zoonotic transmission events. Such ongoing transmission currently exists, given continued Middle East respiratory syndrome coronavirus (MERS-CoV) spillover from camels to humans. Although current research focuses on tackling the COVID-19 pandemic, there is a need to broaden our wildlife surveillance efforts to identify viruses with zoonotic potential.

Ecological factors may also promote the spillover of wildlife-borne viruses. For example, nutritional and reproductive stresses are associated with increased Hendra virus replication in bats [15]. Factors such as urbanization, deforestation and forest fragmentation, mixed farming practices, and other anthropogenic interference with wildlife habitats may indeed alter the delicate balance that reservoir species have evolved with their viruses. In addition, animal stress from unsustainable livestock industries, wildlife trade, and artificial co-housing of different animal species provides pathogens with the opportunity to find novel alternative hosts that are unlikely to occur in a natural setting. Indeed, investment in science with a One Health focus will tackle future emerging zoonoses and closely monitor ecosystem health to limit human interference and minimize exposure across the human–wildlife interface.

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Forum

Animal Behavioral Responses to the COVID-19 Quietus

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Lockdown measures fundamentally reshaped human society during the COVID-19 pandemic. We present a framework featuring seven animal behavioral changes as a result of the calming effect of the lockdowns on human actions (COVID-19 quietus). We demonstrate how this framework can be used to quantify animal behavioral responses with implications for ecology and conservation.

The Coronavirus Disease 2019 (COVID-19) Quietus

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes COVID-19, began to spread person-to-person in a wet market in Wuhan, China in December 2019. Given the high degree of global human interconnectedness, the disease followed transportation networks and was classified as a pandemic by the World Health Organization on 11 March 2020. Between March and July 2020, >100 countries around the world implemented some version of a lockdown, with subsequent national and regional lockdowns occurring periodically thereafter. Restrictive measures included banning large public gatherings, closing educational institutions, strictly regulating travel, and isolating people in their homes. Consequently, the organization of human society around the world fundamentally changed and the spatio-temporal intensity of human activities calmed. Correspondingly, we refer to these phenomenological changes as the 'COVID-19 quietus'. Though quietus is a word with many meanings, including a release from debt or indeed from life, we use it here to describe, as the Oxford English Dictionary defines it, 'a quiet period' or 'a time of inactivity'.

Unintentionally, the COVID-19 quietus provided a quasi-experiment by which to measure the recovery of the natural world to fundamental changes in human behavior. Ecological analyses immediately began describing the impact of the quietus on Earth's abiotic characteristics. The consumption of fossil fuels, for instance, reached 25-year lows in the COVID-19 quietus, with US oil prices plunging into the negative for the first time in history [1]. Accordingly, the daily global CO_2 emissions reduced by 17% during the quietus when compared with rates from the previous year [2]. Shortly thereafter, studies began to emerge describing changes in animal behavior in response to the COVID-19 quietus. White-crowned sparrows (*Zonotrichia leucophrys*) in the San Francisco Bay area of California, United States, for instance, responded to swift reductions in anthropogenic noise by shifting their song volume and structure to maximize communication distance [3]. Eastern cottontail rabbits (*Sylvilagus floridanus*) in Italy were significantly more active diurnally when compared with previous years [4]. Furthermore, several species of carnivore explored urban areas in Chile, though it was unclear whether these observations could be attributed to the quietus [5]. We highlight here that detecting changes in animal behavior in response to the COVID-19 quietus should be expected. Such changes, however, may not be particularly influential unless they have corresponding impacts on animal survival and reproduction [6]. Therefore, quantifying the costs and benefits of these animal behavioral responses is integral to determining the impact of the COVID-19 quietus on animal ecology and conservation. To facilitate such studies, we developed a typological framework of animal behaviors that could reasonably be expected to vary in response to the COVID-19 quietus.

Typology of Animal Behavioral Responses

Our typological framework includes animal: (i) activity schedules, (ii) density, (iii) exploratory behaviors, (iv) movement dynamics, (v) ranging and resource use, (vi) vocalizations, and (vii) vigilance (Figure 1). We highlight that the boundaries between these typologies should not be envisioned to be mutually exclusive. On the contrary, we should expect changes in animal behavior across numerous typologies. For instance, an animal might expand their exploratory behaviors in the COVID-19 quietus with coupled changes in activity schedules and movement dynamics. Quantifying the precise impacts of the COVID-19 quietus on animal behavior will depend on comparison of these typologies across spatial and temporal extents.